The leaf anatomy of two *Clerodendrum* species (Verbenaceae)

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The leaf anatomy of *Clerodendrum triphyllum* (Harv.) H.Pearson and *C. louwalbertsii* P.P.J.Herman is described. The leaves are amphistomatic, with mostly diacytic but also anisocytic, anomocytic and a few paracytic stomata. In cross section the leaves of *C. louwalbertsii* are dorsiventral but in *C. triphyllum* the mesophyll is homogeneous. The leaf surface of *C. louwalbertsii* is smooth, bulgy and undulate, whereas the surface of *C. triphyllum* is reticulate and less bulgy. Peltate hairs are scattered over both leaf surfaces of both species. The hairs have an 8-celled head, a unicellular stalk and a base cell which is sunken below the level of the epidermis.

Keywords: Anatomy, *Clerodendrum*, homogeneous, leaf surface.

Introduction

Clerodendrum L. is probably the largest genus in the family Verbenaceae (Moldenke 1973), with approximately 560 species and varieties distributed mostly in Africa and Asia. Only about 20 species are native to the New World and many are cultivated as ornamentals (Rueda 1993). The genus is represented by about 21 species in southern Africa (Herman 1993), varying from trees and shrubs to perennial herbs.

During preparation for a previous work (Retief & Herman 1997), the material of *inter alia Clerodendrum triphyllum* (Harv.) H.Pearson housed in the National Herbarium of the National Botanical Institute, Pretoria (PRE) was studied, and it became clear that there was more than one taxon represented in the collection. *C. louwalbertsii* P.P.J.Herman was subsequently described as a new species (Herman 1995). The leaf morphology, as reported here, was studied as additional support for the recognition of two taxa.

Materials and Methods

Live material of *C. triphyllum* was collected in natural grassland in the Pretoria National Botanical Garden and of *C. louwalbertsii* at Meintjieskop, Pretoria. Voucher specimens were donated to the National Herbarium, Pretoria (PRE). Material was fixed in FAA (Johansen 1940) or 0.1 M phosphate buffered solution (pH 7.4) of 5% formaldehyde (Collins & MacNichol 1978); 0.5% caffeine was added to the buffered solution, according to the method of Mueller and Greenwood (1977). Pieces from the middle of the leaf were embedded in wax and sectioned at 7 to 10 µm on a rotary microtome. Pieces were also dehydrated, infiltrated and embedded in glycol methacrylate (GMA) (Feder & O'Brien 1968) and sectioned at 2–3 µm on a Jung PM 2045 microtome. Wax sections were stained in safranin and fast green, while GMA sections were stained with the periodic acid/Schiff's (PAS) reaction and counter-stained with toluidine blue (Feder & O'Brien 1968). The sections were studied with an Olympus Vanox-S microscope and photographed using Ilford Panf black-and-white film (ASA 50). Leaf epidermis was obtained by maceration in Jeffrey's solution (Kiger 1971); it was then stained in a 1% aqueous safranin solution and mounted in Entallen. Pieces of dried leaf material were sputter-coated with gold and studied under an ISI SX 25 Scanning Electron Microscope (SEM).

Observations

Light microscopy

Epidermis

The epidermal cells are quadrangular to rectangular in transverse section. Above and below the main vein the cells are smaller than over the rest of the leaf. The leaves are amphistomatic (Figure 1A and B). The stomata occur in the same plane as the rest of the epidermal cells (Figure 1A, B and D) and are mostly diacytic (Figure 1C) but anisocytic, anomocytic and a few paracytic stomata were also observed. Peltate hairs occur in both the adaxial and abaxial epidermis. They consist of a basal cell, sunken below the level of the epidermis, a unicellular stalk and 8-celled head (Figures 1D, E and 2D). In epidermal preparations, epidermal cells radiating around the base of the peltate hairs were observed, more pronounced in *C. triphyllum*. In surface view the epidermal cells above and below the main and first order veins are rectangular and arranged in parallel rows. This arrangement is also characteristic of the epidermal cells along the leaf margin, but the cells are more or less isodiametrical and regular. The intercostal epidermal cells are irregular, vary in shape with straight cell walls (Figure 1C). The cuticle of *C. triphyllum* seems thicker than that of *C. louwalbertsii*.

Mesophyll

The leaves of *C. louwalbertsii* are dorsiventral with the mesophyll distinctly divided into palisade and spongy parenchyma (Figure 1A). In *C. triphyllum* the mesophyll is more homogeneous, not clearly divided into palisade and spongy parenchyma (Figure 1B). The mesophyll of *C. triphyllum* is fairly tightly packed, with fewer intercellular spaces than in *C. louwalbertsii*. Numerous substomatal air chambers are present below the adaxial and abaxial epidermis of both species. In cross section the leaves of *C. louwalbertsii* seem thinner than those of *C. triphyllum*. Collenchyma is present at the leaf margin of both *C. triphyllum* and *C. louwalbertsii*.

Venation

The main vein in the leaves of both species is raised abaxially. Collenchymatous tissue is present below the adaxial and abaxial epidermis above and below the main vein in both species (Figure 1F). This collenchymatous tissue is separated from the main veins by thin-walled isodiametric cells and a thin-walled bundle sheath. In cross section the main veins of both species are arc-shaped with two or three additional vascular bundles adaxially. The main vein of *C. triphyllum* is sometimes cylindrical, with no additional adaxial vascular bundles. The first-order veins of both species are surrounded by a thin-walled bundle sheath. Sometimes a few collenchymatous cells occur adaxially of the first-order veins of *C. louwalbertsii* below the epidermis, but they are not continuous with the bundle sheath. Some collenchymatous cells sometimes occur abaxially of the first-order veins of *C. triphyllum* below the epidermis, not continuous with the bundle sheath.

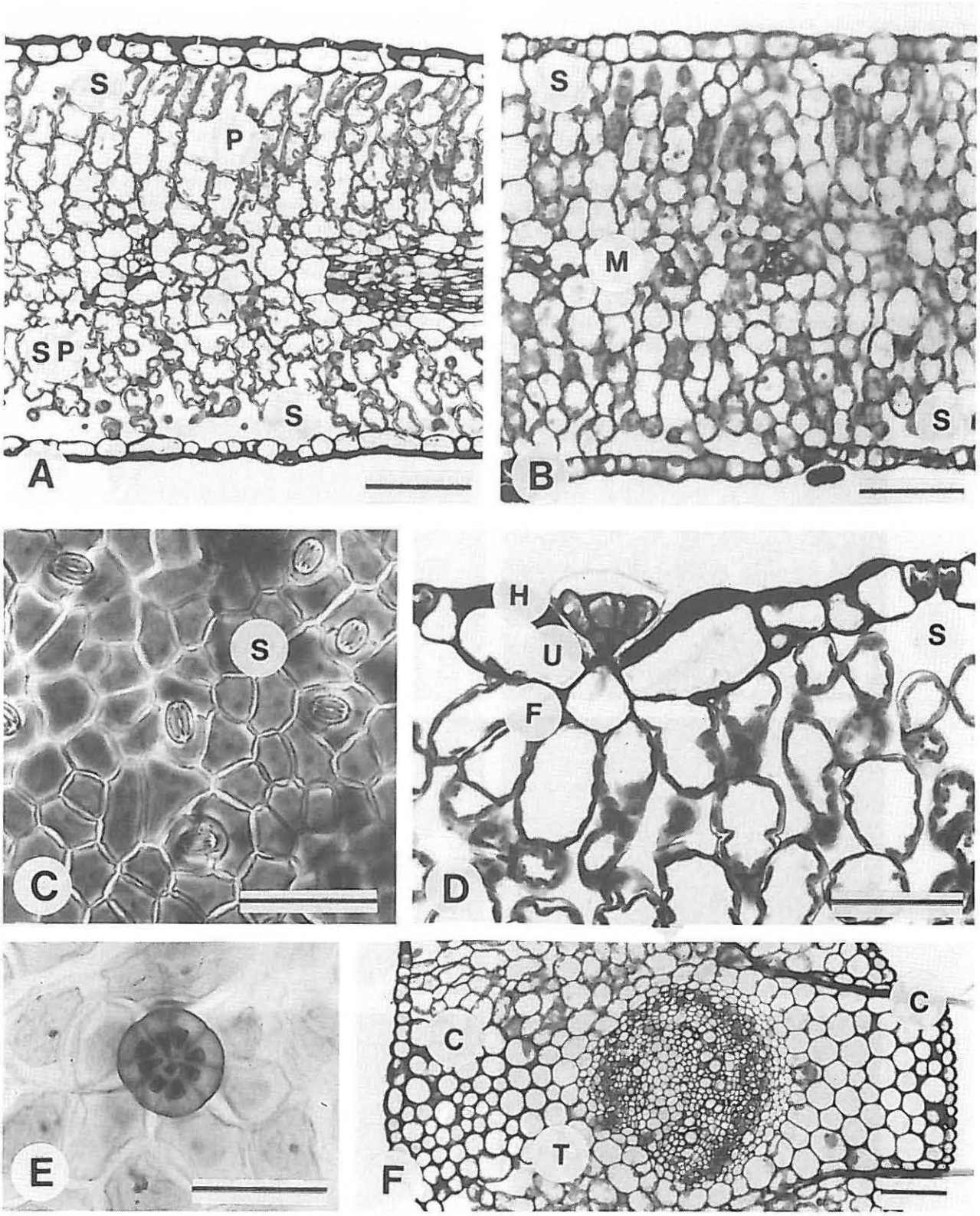

Figure 1 Light micrographs of the leaf anatomy of the two *Clerodendrum* species. **A**. Cross section through the leaf of *Clerodendrum louwalbertsii* to illustrate the stomata (S) in both the adaxial and abaxial epidermis and the clearly divided palisade (P) and spongy (SP) parenchyma of the mesophyll. Scale bar = 100 µm. **B**. Cross section through the leaf of *C. triphyllum* to illustrate the stomata (S) in both the adaxial and abaxial epidermis and the homogeneous mesophyll (M). Scale bar = 100 µm. **C**. Epidermal preparation of *C. louwalbertsii* to illustrate the diacytic stomata (S) and irregular intercostal epidermal cells. Scale bar = 100 µm. **D**. Cross section through a peltate hair in the epidermis of *C. louwalbertsii* with basal cell (F), unicellular stalk (U) and multicellular head (H); (S) stoma. Scale bar = 50 µm. **E**. Epidermal preparation of *C. louwalbertsii* to illustrate the 8-celled head of the peltate hair. Scale bar = 50 µm. **F**. Cross section through the main vein area of *C. triphyllum* to illustrate the collenchyma (C) below the adaxial and abaxial epidermis and the thin walled tissue (T) around the vascular bundle. Scale bar = 100 µm.

Scanning Electron Microscopy (SEM)

In surface view the leaf surface of *C. louwalbertsii* shows a bulgy, undulate pattern (Figure 2A). The leaf surface of *C. triphyllum* seems less bulgy and smoother (Figure 2B). In both species the leaf margin is recurved and the surface along the margins has an elongated pattern as above the main vein, especially obvious on the abaxial surface (Figure 2C). The peltate hairs are clearly visible under the SEM (Figure 2D). Under high magnification, the surface of *C. louwalbertsii* is quite smooth (Figure 2E) except for occasional striations at the base of the peltate hairs and occasionally around the stomata. In *C. triphyllum* the surface has a 'dry mud' (reticulate) appearance (Figure 2F). No significant differences were observed in the stomata and peltate hairs of the two species.

Discussion

Kereszty (1993–94) studied the leaves of various *Clerodendrum* species under the SEM and found significant differences, in especially the lower surface and the stomata, to separate species and infraspecific taxa. In the present study, significant differences were observed under the SEM to support the recognition of two separate taxa: the smooth, bulgy, undulate leaf surface of *C. louwalbertsii* and the reticulate, less bulgy leaf surface of *C. triphyllum*.

Solereder (1908), Metcalfe and Chalk (1950), Metcalfe (1979) and Metcalfe and Chalk (1979) reported homogeneous mesophyll and dorsiventral leaves in the family Verbenaceae. In *C. louwalbertsii* the leaves are dorsiventral, but in *C. triphyllum* the mesophyll is homogeneous. However, it was surprising to find two different types of mesophyll arrangement in two species of the same genus with similar habitats (grassland) and life forms (perennial herbs), as observed in this study.

Several authors have described the peltate hairs found in many members of the family Verbenaceae (Solereder 1908, Metcalfe & Chalk 1950, Inamdar 1969, Theobald *et al.* 1979, Abu-Asab & Cantino 1987, Cantino 1990 & Kereszty 1993–94). The general

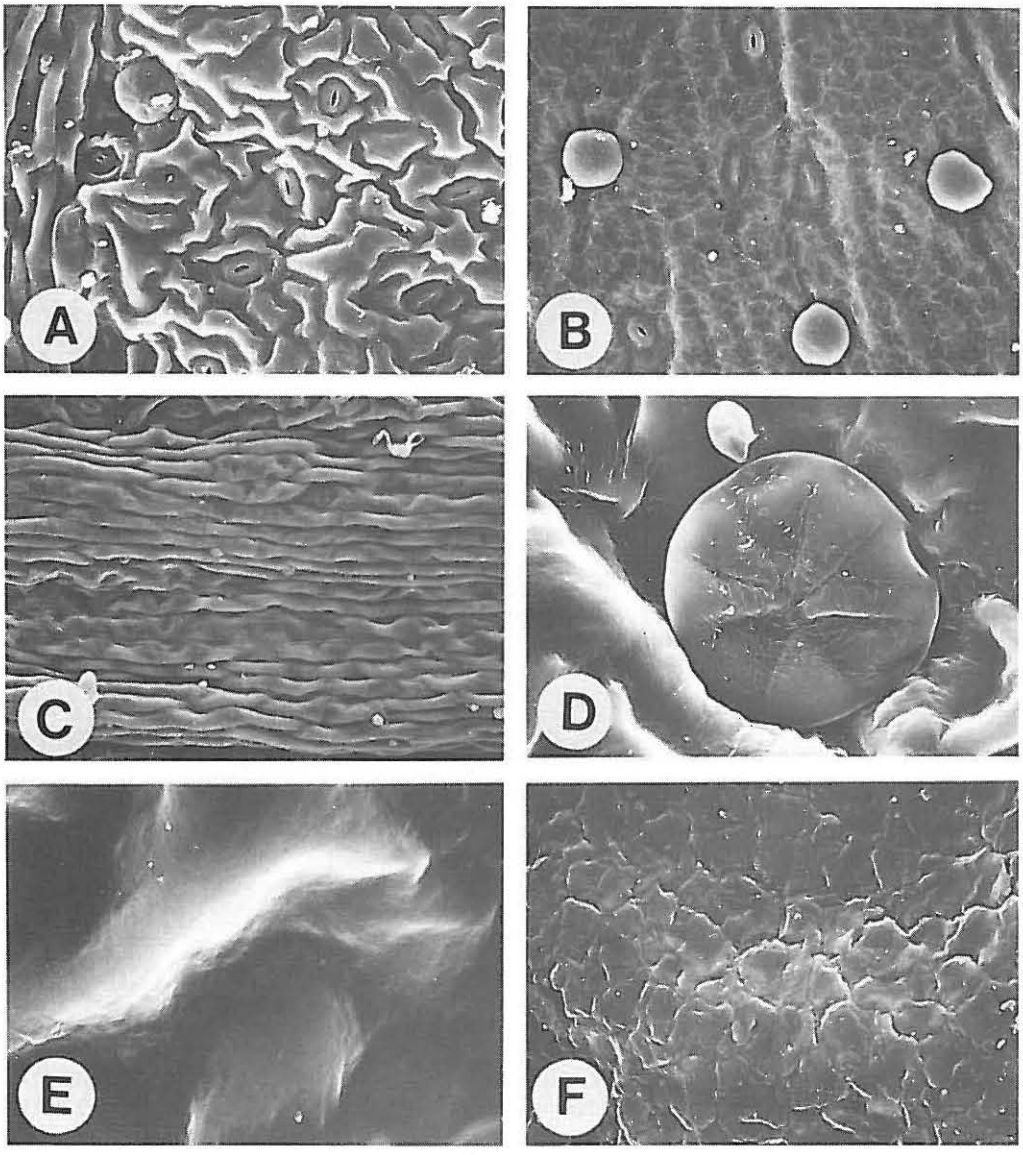

Figure 2 Scanning electron micrographs of the leaf surfaces of the two *Clerodendrum* species. **A**. Adaxial leaf surface of *C. louwalbertsii* to illustrate the bulgy, undulate pattern, × 210. **B**. Adaxial leaf surface of *C. triphyllum* to illustrate the less bulgy pattern, × 210. **C**. Abaxial leaf surface of *C. louwalbertsii* to illustrate the elongated pattern along the main vein, × 137.4. **D**. Adaxial leaf surface of *C. louwalbertsii* to illustrate the 8-headed peltate hair, × 726. **E**. The smooth abaxial leaf surface of *C. louwalbertsii* under high magnification, × 1380. **F**. Abaxial leaf surface of *C. triphyllum* to illustrate the 'dry mud' (reticulate) appearance under high magnification, × 1380.

morphology of the peltate hairs present in the two species studied was basically the same as that described by these authors, and no significant differences were observed between the peltate hairs of the two species: in both cases the hairs had an 8-celled head, a unicellular stalk and a base cell.

The stomata in the amphistomatic leaves of *Clerodendrum triphyllum* and *C. louwalbertsii* are diacytic, anisocytic, anomocytic and paracytic according to the classification system of Dilcher (1974) and Wilkonson (1979). Among the Cuban *Clerodendrum* taxa, Kereszty (1993–94) found only hypostomatic leaves and anomocytic, actinocytic, anisocytic and paracytic stomata. Inamdar (1969) also found only hypostomatic leaves but mostly diacytic stomata in the Indian *Clerodendrum* taxa studied by him. Cantino (1990) reported 3-celled diallelocytic stomata in all the species of *Clerodendrum* subgenus Cyclonema he examined. *C. triphyllum* and *C. louwalbertsii* both belong to the subgenus Cyclonema (Verdcourt 1992) but only diacytic, anisocytic, anomocytic and paracytic stomata were observed during the present study.

Conclusion

Significant differences observed in leaf surface structure as seen under the SEM and mesophyll structure, support the recognition of two distinct species, *C. triphyllum* and *C. louwalbertsii*.

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